

The Advantages of Chlorophyll Extraction with Alcohol

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Laboratory studies of the chlorophylls and other plant pigments are very common exercises in general biology and botany courses. Nearly all published procedures specify disruption of leaf tissue with a mortar and pestle or high speed macerator (usually a blender) and extraction of the pigments in acetone. Because acetone is an extremely dangerous chemical, I suggest the use of an equally effective, yet much less dangerous solvent—ethyl or methyl alcohol.

Acetone (2-propanone) at room temperature is an extremely volatile, highly flammable liquid with a dangerously low flash point of -20°C (Merck Index 1976a). In an open container, liquid acetone readily vaporizes and is even more hazardous in the gaseous state. The Merck Index (1976a) warns that this solvent must be kept away from fire or sparks and that it will damage plastics, jewelry, pens, rayon and clothing. Moreover, the Index cautions that repeated or prolonged exposure to acetone may cause skin problems, and inhalation of vapors causes headache, nausea, fatigue, and irritation and damage to the respiratory system. Exposure to large amounts of acetone vapor causes narcosis and drinking the liquid may be fatal. I have witnessed two separate accidents involving acetone vapors ignited by sparks or friction from laboratory equipment.

One flash fire was caused by an electric food blender during chlorophyll extraction in acetone. Both accidents resulted in severe burns to laboratory workers. Acetone should only be used in a fume hood

equipped with safety devices such as a vapor-proof light.

Ethanol (ethyl alcohol) is less volatile than acetone and has a higher flash point of 9 to 11°C (Merck Index 1976b). Though this solvent is much less dangerous than the acetone, careful handling is still necessary as ethanol is flammable and toxic (Merck Index 1976b). Denatured alcohol is a slightly less satisfactory alternative solvent than ethanol for two reasons; first, some plant pigments are less soluble in cold methanol than ethanol (Merck Index 1976c); second, methanol is poisonous and somewhat more hazardous to human health than ethanol (Merck Index 1976d). Fire hazard with the two alcohols is similar and the flash point of methanol is 12°C (Merck Index 1976d). Economics and availability often dictate the choice of alcohol, but I have had better success with ethanol.

Holden (1965) stated that the efficiency of extraction with methanol and acetone was about the same. I found this was also true with commercial 95% (by volume) aqueous ethanol and prepared 80% (by volume) aqueous acetone (these are commonly employed concentrations). A one-pound (454 g) package of frozen spinach was extracted in 1 liter of each solvent by maceration in a blender for several minutes. Each dark green extract was diluted to 2:1; the original solvent was then passed through filter paper under negative pressure in a Büchner funnel. Yield was measured spectrophotometrically (Holden 1965) and found to be 0.95 mg chlorophyll/g spinach with acetone and 0.89 mg

chlorophyll/g spinach with ethanol, a negligible difference for teaching-laboratory exercises. Similarly close yields were obtained with fresh spinach and leaves of other common greenhouse plants. Less concentrated alcoholic solutions (i.e., 80%) were not as efficient for extraction and problems were encountered during filtration. In my laboratory tests, rubbing alcohol (70% isopropyl alcohol) was significantly inferior to the above solvents for extraction of chlorophylls from leaves of several plants. (See Klein 1979.)

Some laboratory manuals suggest boiling green leaves in solvent to extract the chlorophylls. This practice would be dangerously foolish with acetone and is not necessary with alcohol. Boiling actually accelerates the breakdown of chlorophylls to pheophytins, pheophorbides, and other poorly described brownish compounds; thus, extraction with cold solvent may be preferable (Holden 1965). In general, chlorophylls are quite labile and isomerize or decompose spontaneously in solution especially at elevated temperatures and extreme pH, or when in the presence of strong oxidizing agents (Strain and Svec 1966). Exposure to intense light is also harmful to chlorophyll solutions (Strain and Svec 1966). Maceration, extraction, and filtration should be done quickly and at low temperatures; CaCO_3 , MgCO_3 , NaHCO_3 or Na_2CO_3 (about 100 mg/l) may be added to the solvent to prevent pheophytin formation during maceration (Holden 1965). Final alcohol content should be above 90% (by volume) to prevent chlorophyllase activity in

extracts of fresh tissue (Holden 1965). Subsequent experiments with the chlorophylls should be done as soon as possible after extraction, but the extract may be stored for short periods (less than four days in most cases) in a cold, dark place.

Following extraction, the plant pigments are often studied further in numerous experiments described in most introductory laboratory manuals. For example, determinations of chlorophyll concentrations and absorption spectra with a spectrophotometer (see Kaufman, *et al.* 1975, Holden 1965), or separation of the pigments by paper chromatography (i.e., BSCS laboratory exercises and many other general biology and botany manuals; see also Klein 1979) are often done. Alternatively, I suggest the less often used but classic and more striking, yet simple, separation of plant pigments on a column of sugar. These exercises may be found in Kaufman *et al.* (1975), Strain and Svec (1966) and other laboratory manuals.

Prior to the above exercises, the alcoholic extract (454 g/2 l) may be used in an impressive demonstration to introduce the topic of photosyn-

thesis. Fill a one-liter graduated cylinder with fresh, green extract. Turn off the classroom lights and shine bright, white light from a single source (a desk lamp with 75 or 100 watt bulb works well) on the cylinder. If students surround you in a circle during this demonstration, some will see the solution as dark green and some as dark red as the chlorophyll will exhibit a deep-red fluorescence. Tilting the cylinder will cause a change in which of the two colors the students perceive. At this point, I ask my students to formulate a hypothesis to explain what is occurring *in vitro* and then relate it to *in vivo* (when chlorophyll in solution is excited by the light there is no active coupling mechanism for using the energy that is dissipated as heat and emitted as lower-energy red light in the visible spectrum). The success of this demonstration depends on the proper concentration of chlorophyll in solution and the correct light intensity; both of which may be easily determined empirically.

In conclusion, chlorophylls and other plant pigments may be as efficiently yet more safely extracted in alcohol than in acetone. Alcoholic

extracts may be effectively used for subsequent studies on the properties of the pigments.

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Team Games for Biology Students

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When you want to review for a test in biology or go over material in a chapter, why not do it in a different way by using a game? Learning biology can be fun. When learning is fun, the classroom climate and attitudes of students toward learning improve. When attitudes toward a subject and toward learning improve, the students' desire to learn will grow. I have used a variety of games with my classes to create more interest in biology, and I have found that fast-paced team games keep all my students attentive and involved. Here

are four games I have used successfully.

"Upset the Apple Cart"

Divide the class into four or five teams, and seat the students in rows. Provide the student at the front of the row with a sheet of paper and a pencil for writing answers. When the teacher asks a question, the student at the front of the row quickly writes the answer and rushes it to the teacher. S/he may receive help from those students seated behind who whisper the answer to each other and pass it to the front of the row. The team that gets the correct answer to the teacher wins a point. Questions should be fired as rapidly as possible by the

teacher. After about ten questions, the teacher says, "Upset the apple cart." On this command, the student at the front of the row moves to the back, and all the other students shift forward one seat. Ten or more questions are again asked rapidly with students at the front of the rows taking their answers to the teacher. Again the command is given to "Upset the apple cart," and all the students shift seats. As the game continues, all the students should have the opportunity to be at the front of the row. The game can be played for an entire class period to review a complete chapter. Points are tallied at the end of the game to determine the winning team.